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Electronic Supplementary Information

of

Metal-semiconductor nanocomposite as an efficient oxygen-independent photosensitizer for photodynamic tumor therapy

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Materials. Cadmium oxide (CdO), dodecylamine (DDA), sulfur (S) were purchased from Shanghai Reagent Chemical Co. (Shanghai, China) and used directly. N,N-Dimethylformamide was purchased from Shanghai Chemical Co. (Shanghai, China) and used after redistillation. Tetrachloroauric acid ($HAuCl_4$•$4H_2O$) was purchased from Sigma-Aldrich (USA). N-hexylphosphonic acid (HPA) was purchased from Strem Chemicals Inc. Selenium (Se) was purchased from Kemiou Chemical Reagent Co., Ltd.. N-Octadecylphosphonic acid (ODPA) was purchased from Sigma-Aldrich (USA). Trioctylphosphine oxide (TOPO) was purchased from TCI chemicals (Shanghai, China). Tetraoctylammonium bromide (TOAB) was purchased from Heowns Biochemical Technology Co., Ltd.. N-fluorenyl-9-methoxycarbonyl (Fmoc) protected amino acids, O-benzotriazole-N,N,N′,N′,-tertamethyluroniumhexafluorophosphate (HBTU), N-hydroxybenzotriazole (HOBt), Rink-Amide resin (100-200 mesh, loading: 0.51 $mmol \cdot g^{-1}$) were purchased from GL Biochem Ltd. (Shanghai China). Annexin V-FITC/PI apoptosis and necrosis detection kit was purchased from 4A Biotech Co., Ltd (Beijing, China). Diisopropylethylamine (DIEA), Trioctylphosphine (TOP), Trifluoroacetic acid (TFA) and Piperidine were purchased from Energy-Chemical. Triisopropylsilane (TIS) was purchased from Sigma-Aldrich (USA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT), Dulbecco′s Modified Eagle′s Medium (DMEM), Fetal Bovine Serum (FBS), the micro BCA protein assay kit, Penicillin-streptomycin and Dulbecco′s phosphate buffered saline (PBS) were purchased from Pierce. All other chemicals were of analytical grade and used as received directly.

Characterization. Transmission electron microscopy (TEM) images were obtained on JEM-2100 (JEOL) transmission electron microscope. Confocal laser scanning microscope (CLSM) images were obtained on a C1-Si (Nikon) confocal laser scanning microscope. FT-IR spectrum was recorded on a Spectrum Two FT-IR Spectrophotometer (Perkin-Elmer). Zeta potentials and particle size were measured by a zeta sizer (Nano ZS, Malvern Instruments). Blood routine was examined by Auto Hematology Analyzer (MC-6200VET). Blood biochemistry analysis was measured by biochemical auto analyzer (MNCHIP, Tianjin, China).

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Synthesis of CdSe Quantum Dot (QDs). 3 g trioctylphosphine oxide (TOPO), 280 mg octadecylphosponic acid (ODPA) and 60 mg cadmium oxide (CdO) were mixed in a 25 mL three-neck flask. The mixture was heated to 150 °C and kept the vacuum for 30 min and then turned to Ar atmosphere. After that, the solution was heated (270 °C) to dissolve the CdO until it turned optically clear and colorless. At this point, 1.5 mL of TOP was rapidly injected into the flask. When the temperature was raised to 360 °C, the Se/TOP solution (0.058 g Se in 1.2 mL TOP) was swiftly injected into the flask. After 3 min, the solution was removed from heat and cooled to room temperature. Then 10 mL toluene was added to the solution, which was washed three times with methanol and re-dispersed in 20 mL toluene for further use.

Synthesis of CdSe-Seeded/CdS Nanorods (NRs). Synthesis of NRs *via* seeded growth, 3 g TOPO (99%), 65 mg CdO, 290 mg ODPA and 80 mg hexylphosphonic acid (HPA) were mixed in a 25 mL three-neck flask. The mixture was heated to 150 °C and exposed to vacuum for 30 min and then heated to 360 °C under Ar atmosphere. When the solution turned from reddish brown to colorless, 1.5 mL of TOP was rapidly injected into the flask. When the temperature was recovered to 360°C, a mixture solution of S/TOP and CdSe seeds were obtained by first dissolving 80 mg S in 1 mL TOP at 50 °C before adding 1 mL of the prepared CdSe/TOP solution (which was redissolved in 1 mL TOP after removed the toluene), and was swiftly injected into the flask. Upon reaching the expectant temperature, 1.8 mL TOP was added, and the temperature was allowed to recover to 360 °C before the mixture of S/TOP and CdSe seeds were swiftly injected. The nanocrystals were allowed to grow for about 8 min after the injection. The temperature was again allowed to recover to 360 °C in 3.5 min and keep growing at this temperature for 4.5 min. The heat was then removed and the solution was cooled to room temperature. Then 10 mL toluene was added to the solution, which was washed three times with methanol and re-dispersed in 20 mL toluene for further use.

Synthesis of CdSe-Seeded/CdS@Au Hybrid Nanocomposites (HNCs). Separately, 1 mL of Au stock solution(13 mg $HAuCl_4 \cdot 4H_2O$ and 17.6 mg tetraoctylammonium bromide (TOAB)) and 1 mL of DDA solution (28 mg dodecylamine (DDA) in 1 mL toluene) were mixed in vial A and sonicated at room temperature for 15 min to give a light yellow solution. In a separate vial B, the mixture was derived by mixing of 1 mL of a solution of CdSe-seeded/CdS NRs as prepared and 12 mg of ODPA. Under Ar atmosphere, the solution of vial A was swiftly injected into the flask after the solution of vial B was added into the 10 mL three-neck flask and degassed. This mixture was stirred vigorously under Ar atmosphere at room temperature. The reaction was allowed to proceed for an adequate time, typically 25 min and then quenched with methanol, afterthat, the solution was washed three times with methanol and re-dispersed in toluene for further use.

Synthesis of PEG-RGD. PEG-RGD was synthesized *via* solid-phase synthesis. Briefly, the peptide was conjugated to a rink amide resin and HBTU/HOBT/DIEA were used to promote coupling efficiency of the Fmoc-protected amino acids. After the solid-phase synthesis, the product was cleaved from the resin by using an efficient cleavage cocktail of TFA, DI water, and TIS (volume ratio of 95:2.5:2.5) for 1.5 h. The molecular weight of PEG-RGD was determined by electrospray ionization mass spectrometry (ESI-MS) system (Finnigan LCQ Advantage).

Synthesis of HNCs-RGD. Synthesis of HNCs-PEG-RGD *via* ligand exchange, 10 mg prepared PEG-RGD was dissolved in 20 mL methanol. The RGD solution was delivered into the 50 mL centrifuge tube after the toluene was removed from the HNCs solution. The turbid solution turned to clear solution after sonicated at room temperature in a pH of 8. Then, the mixture was stirred at room temperature for 30-60 min to complete the ligand exchange. Finally, the HNCs-PEG-RGD was obtained through centrifugation at 10000 rpm for 30 min. In order to obtain purified HNCs-REG-RGD, the mixture was purified with ultra-pure water using centrifugal filters for 2 days and then lyophilized for further use. The content of Cd element was quantified by ICP-AES.

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TEM and EDX Characterization. A JEOL 2100 (200 kV) microscope was used to conduct the TEM characterization of QDs, NRs and HNCs by dropping the sample solution on copper grids and the drop was dried at room temperature. After morphological observation of QDs, NRs and HNCs, the EDX characterization of HNCs was carried out by Energy Dispersive X-Ray Spectroscopy.

XPS Analysis. The chemical compositions of HNCs were obtained rely on a Thermo Fisher ESCALAB 250Xi multitechnique surface analysis instrument.

UV-Vis Absorption Spectroscopy. The UV-Vis absorption spectroscopy of all samples were gathered using a UV-vis spectrophotometer (Lambda Bio40, Perkin-Elmer). Spectra were studied using the origin software package.

Photoluminescence Measurements. Photoluminescence analysis was carried out on a RF-530/PC spectro fluorophotometer (Shimadzu). The samples were excited at around 450 nm and its emission spectra was measured from 500-700 nm. The spectra were studied using the origin software package.

FT-IR Spectra. FT-IR spectra were obtained by a Perkin-Elmer spectrophotometer (USA) by pressing the samples into potassium bromide (KBr) pellets.

In Vitro ROS Detection by RNO. The generation of ROS was determined by detecting the decrease of UV absorbance of disodium salt of RNO in the prepared solution. Briefly, RNO (100 μL, 5×10^{-5} M) and histidine (200 μL, 6×10^{-5} M) were mixed with 1.7 mL ultima materials solution. The solution was devided into two vials, marked A and B. The solution of A was illuminated under a blue LED lamp (450 nm, 18 W), and the solution in vial B was illuminated after removing the oxygen. The UV absorption at 440 nm (λ_{max} of RNO) was recorded in different irradiation time to monitor the consumption of RNO.

In Vitro ROS Detection by ESR Spectroscopy. ROS were detected by ESR measurements, which were conducted using a Bruker EMX ESR spectrometer (Billerica, MA) at room temperature. 18 mM DMPO was used to demonstrate the production of ROS, mainly hydroxyl radicals (•OH). Five vials were

marked as control, A, B, C and D. Under dark conditions, 500 μL prepared NRs aqueous solution was added to vial A and C, and 500 μL prepared HNCs aqueous solution was added to vial B and D, and 500 μL pure water was added to vial control. Then the same amount of DMPO was injected into the five vials respectively. When the vial C and D were deoxygenated completely, all vials were illuminated for five minutes. After the preparation, Fifty microliter aliquots of prepared mixture were put into quartz capillary tubes and sealed. Then the capillary tubes were inserted in the ESR cavity, and the spectra were recorded during irradiation at selected times. The settings of ESR measurements parameters were as follows: 21.8 mW microwave power, 150 G scan range and 1 G field modulation.

Cell Culture. Cos-7 cells and Hela cells were cultured in DMEM, and 4T1 cells were cultured in 1640 medium containing 10% heat-inactivated FBS and antibiotics (penicillin-streptomycin, 100 U mL^{-1}) at 37 °C with 21% O_2 and 5% CO_2.

Cellular Uptake. To evaluate the cumulative degree of HNCs in normal cells and tumor cells, Cos-7 cells and Hela cells were seeded into a petri dish respectively with DMEM and incubated for 24 h (37 °C, 5% CO_2). After that, the medium was replaced by fresh DMEM contained 2 mg/mL HNCs and incubated for 4 h. Then, cells were washed three times with PBS and observed *via* CLSM (excitation wavelength: 450 nm, emission band pass: 605 nm).

In Vitro ROS Detection by DCFH-DA. The generation of ROS produced by HNCs under visible light irradiation was identified using DCFH-DA (Reactive Oxygen Species Assay Kit) by means of fluorescence spectrum as well as CLSM. To this concern, 1×10^5 4T1 cells/well were seeded into glass bottom cell culture dishes (NEST) and incubated with 50 μg/mL and 25 μg/mL HNCs for 4 h respectively, either under irradiation with a blue light LED or without irradiation. Negative controls were cultured under the same conditions but did not comprise any HNCs treatment. Then, after washed three times with PBS, 1 mL RPMI 1640 medium without FBS contained 10 μM DCFH-DA was added into dishes and incubated for 20 min at 37 °C. The hypoxic groups were managed the same method while 4T1

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cells were cultured in 1640 medium at 37 °C with 1% O_2 and 5% CO_2. Finally, samples of cells were illuminated subsequently (500 mW cm^{-2} for 10 min) and then observed *via* CLSM as soon as possible (excitation wavelength: 450 nm, emission band pass: 605 nm).

Dark and Phototoxicity. The cell toxicity was evaluated by MTT assay. 4T1 cells were seeded into a 96-well plate (5×10^3 cells per well), and then incubated in 100 μL RPMI 1640 medium containing 10% FBS for a 24 h in an incubator (37 ^oC, 5% CO_2). After that, 100 μL fresh RPMI 1640 medium mixed with fixed concentration of HNCs were added into each well. For phototoxicity, after incubated in 37 °C for 4 h, the cells were illuminated by a blue-light LED (500 mW cm^{-2}) for 10 min and further incubated for 20 h as before. Negative controls (dark toxicity) were sostenuto incubated for 24 h at 37 °C. Then, after washed with PBS, the cells were incubated with 200 μL fresh medium contained 20 μL MTT (5 mg/mL) for another 4 h. Subsequently, the medium was removed and 150 μL of DMSO was added. The optical density (OD) was measured at 570 nm with a microplate reader. The relatively cell viability was calculated as follows: viability = (OD_{sample}/ $OD_{control}$) × 100%. The OD_{sample} was obtained from the presence of the agent treated cells, and $OD_{control}$ was obtained from the absence of the agent treated cells.

In Vivo Biodistribution Study. The fluorescence of HNCs-RGD were imaged in organs by IVIS (Spectrum Pre-clinical *In Vivo* Imaging System, PekinEmer) to evaluate the biodistribution of agents *in vivo*. When the tumor volumes of tumor-bearing mice (balb/c, female) reached about 200 mm^3, 200 μg mL^{-1} HNCs-RGD distributed in PBS were injected through tail vein. 6 h and 24 h after the injection, mice were sacrificed respectively and the organs include heart, liver, spleen, lung, kidney and tumor were removed for fluorescence imaging. (Excitation wavelength: 450 nm, emission band pass: 605 nm)

In Vivo Antitumor Treatment.

Animal experiments were carried out according to the guidelines of laboratory animals which were established by Wuhan University Animal Biosafty Level III Lab. All study protocols were approved by

the Institutional Animal Care and Use Committee (IACUC) of the Animal Experiment Center of Wuhan University (Wuhan, China). All mouse experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China. Mice were hypodermically injected with 100 μL 4T1 cells into the hindflank. When the tumors reached an approximate size of 100 mm^3, the mice were divided into five groups randomly and labelled 1 to 5 (n=6). All of the mice were only treated *via* intravenous injection on the first day. The mice of group 1 were treated with 100μL PBS; the group 2 was treated with 100μL NRs-RGD solution (containing 1 mg/mL NRs-RGD); the group 3 was treated with 100μL NRs-RGD solution (containing 1 mg/mL NRs-RGD) and blue light irradiation for 30 min, the group 4 was treated with 100 μL HNCs-RGD solution(containing 1mg/mL HNCs-RGD), the group 5 was treated with 100 μL HNCs-RGD solution (containing 1mg/mL HNCs-RGD) and blue light irradiation for 30 min. The weight of the mice and tumor volume was measured in every two days. Tumor size was measured by a caliper, and tumor volume was calculated as: $V = (\text{tumor width})^2 \times (\text{tumor length})/2$. The mice were sacrificed after sixteen days treatment experiment, and the major organs (heart, liver, spleen, lung, and kidney) and tumors were collected for antitumor mechanism analysis.

In Vivo Blood Biochemistry Analysis and Blood Routine Examination. To evaluate the effects in physiology caused by our differently modified materials, blood biochemical indexes and blood routine indexes were tested *in vivo* respectively. Another 18 female Balb/C mice were randomly divided into 3 groups, which were *i.v.* injected with HNCs (1 mg/kg), HNCs-RGD (1 mg/kg) and control (PBS) (n = 6). After 24 h of post exposure, blood samples were collected from heart (100 μL each mouse). The blood routine was examined by Auto Hematology Analyzer (MC-6200VET), and blood biochemistry analysis was measured by biochemical auto analyzer (MNCHIP, Tianjin, China).

Statistical Analysis. Statistical analysis was performed using a Student's t-test. The differences were considered to be statistical significant for p value < 0.05.

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Table S1. Blood routine indexes of the normal mice treated with HNCs and NRs.

	5 Female mice in each group		
	HNCs	NRs	control
WBC	5.13±1.53	6.10±1.60	3.77±1.13
RBC	9.54±2.03	8.10±1.77	9.65±2.87
HGB	147.33±9.67	135±10.00	147.33±11.67
HCT	45.87±8.63	37.97±7.83	45.2±13.90
MCV	46.7±0.40	46.93±0.67	46.8±0.60
MCH	17.53±4.07	17.1±4.00	13.7±0.80
MCHC	376.67±89.33	364.33±82.33	293±20.00
PLT	225±38.00	328±113.00	281.67±82.67

Compared with control, *p<0.05, **p<0.01, ***p<0.005

Table S2. Blood biochemical indexes of the normal mice treated with HNCs and NRs.

	5 Female mice in each group		
	HNCs	NRs	control
TP	45.60±3.70	47.30±4.30	42.37±0.63
ALB	21.47±1.53	23.97±2.33	20.83±1.67
GLO	24.13±2.17	23.33±2.17	21.43±1.23
A/G	0.90±0.00	1.00±0.10	0.97±0.13
TBIL	2.30±0.20	2.63±0.47*	1.43±0.07
ALT	43.67±14.67	44.67±8.67	50.33±4.67
AST	169±58	112±25	130.67±9.67
GGT	6.23±1.58*	8.19±1.40	10.34±1.31
UREA	4.33±3.67	3.33±1.67	7.00±1.00
GLU	8.80±1.90	13.20±0.50*	10.77±0.53

Compared with control, *p<0.05, **p<0.01, ***p<0.005

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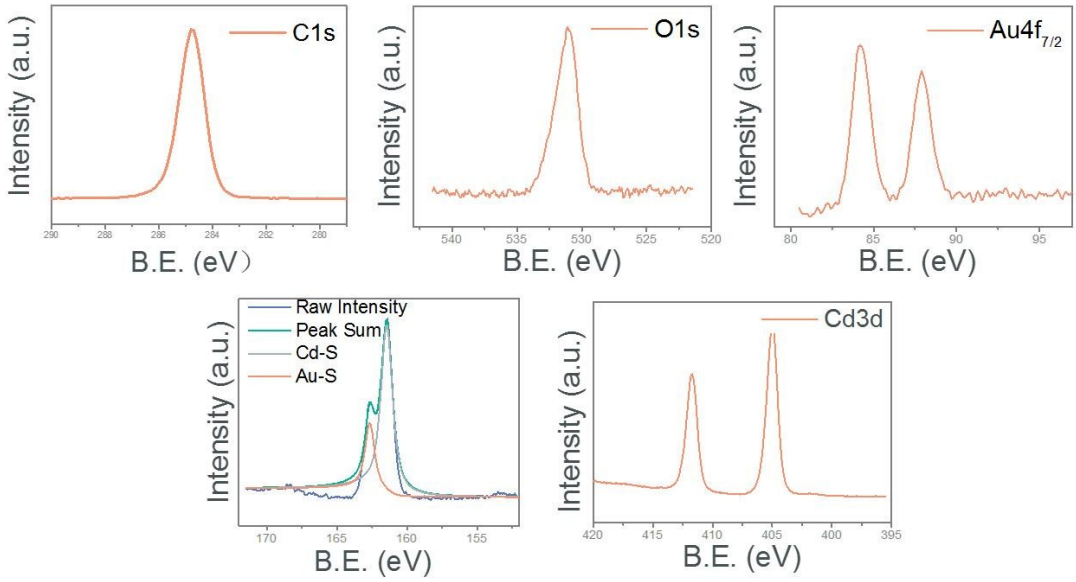

Figure S1. XPS spectrum of HNCs. XPS signal of C1s, O1s, Au4f and Cd3d region.

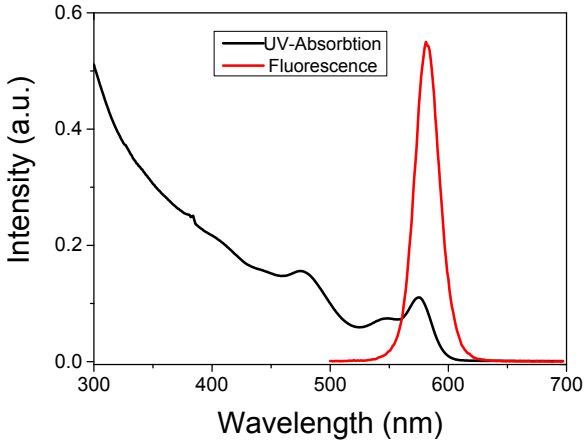

Figure S2. UV-vis absorption spectra and fluorescence of pure QDs.

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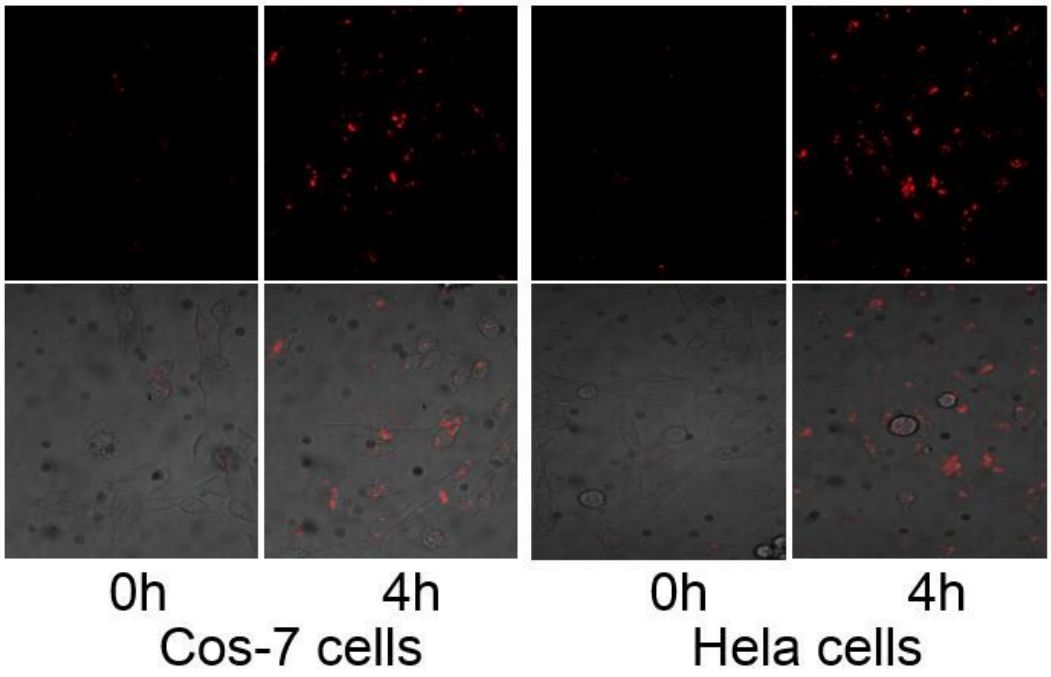

Figure S3. Cellular uptake of HNCs after Cos-7 and Hela cells were incubated with HNCs for 4h.

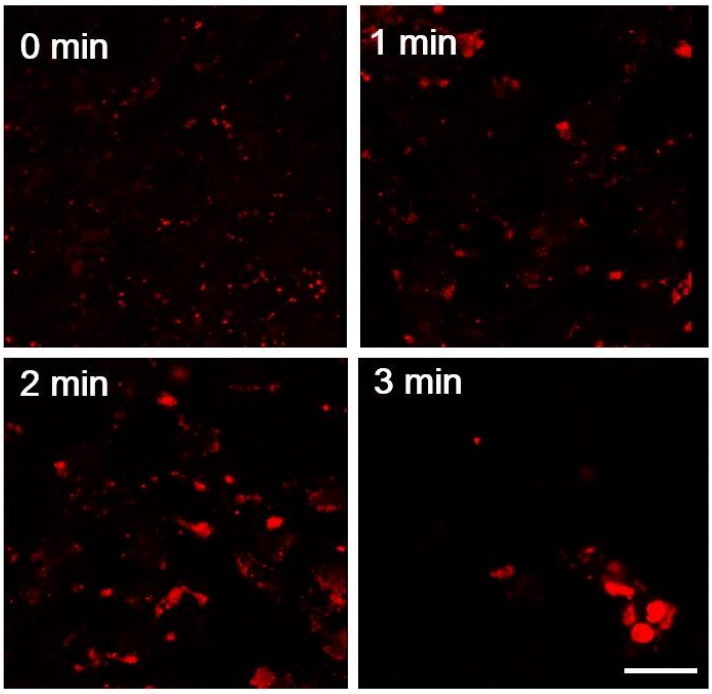

Figure S4. Endocytosis after 4T1 cells were incubated with HNCs and illuminated with blue LED light for different times

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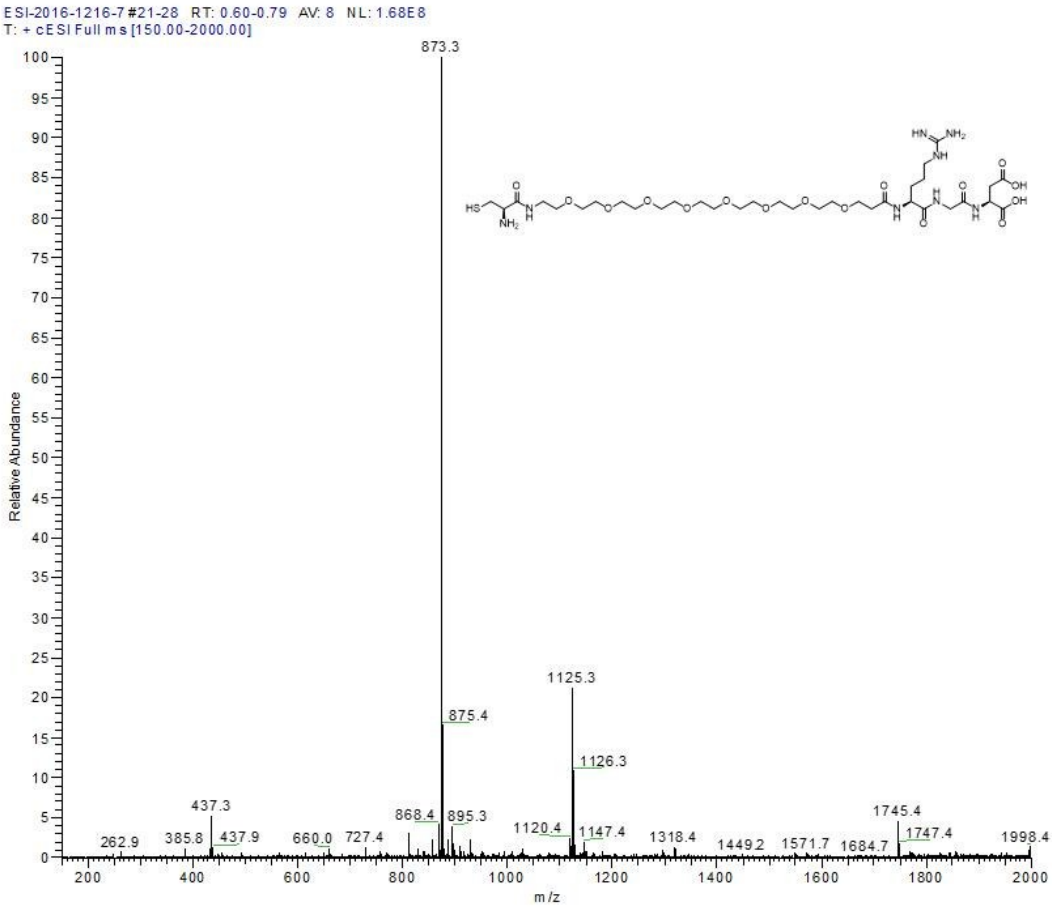

Figure S5. ESI-MS spectrum of C-PEG-RGD.

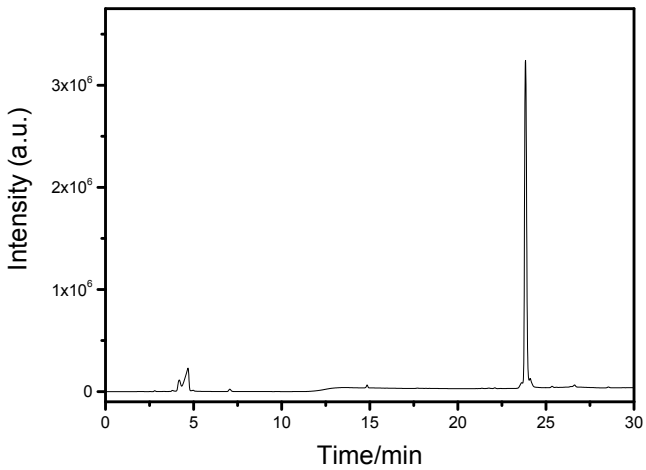

Figure S6. HPLC of C-PEG-RGD**.**

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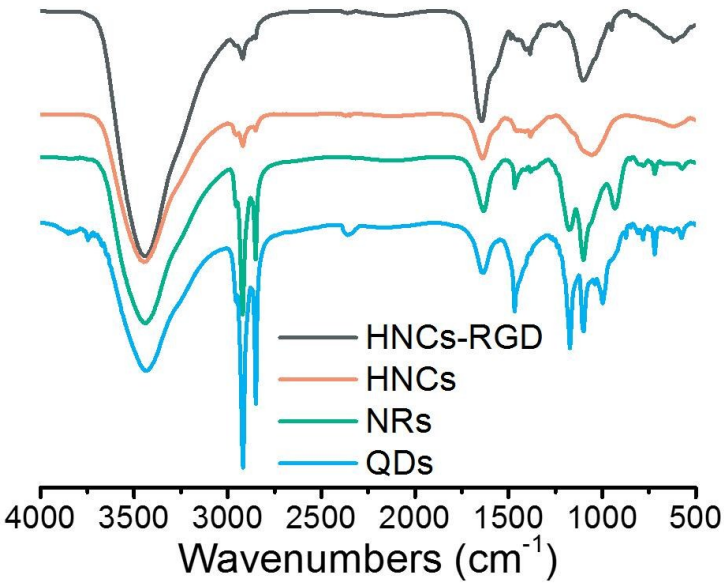

Figure S7. IR spectra of HNCs-RGD, HNCs, NRs and QDs.

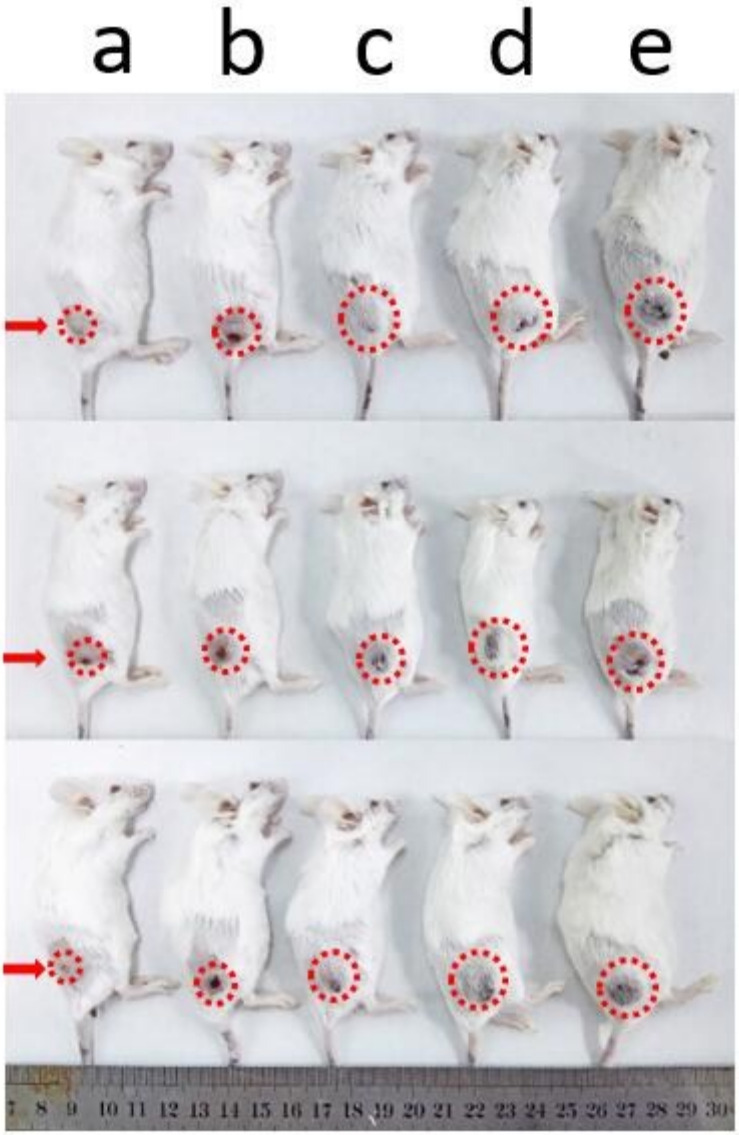

Figure S8. Photographs of 4T1 tumor-bearing mice in different groups after treatment for 16 days. (a) Treated with HNCs and irradiation; (b) treated with NRs and irradiation; (c) treated with HNCs and no irradiation; (d) treated with NRs and no irradiation; (e) treated with PBS.

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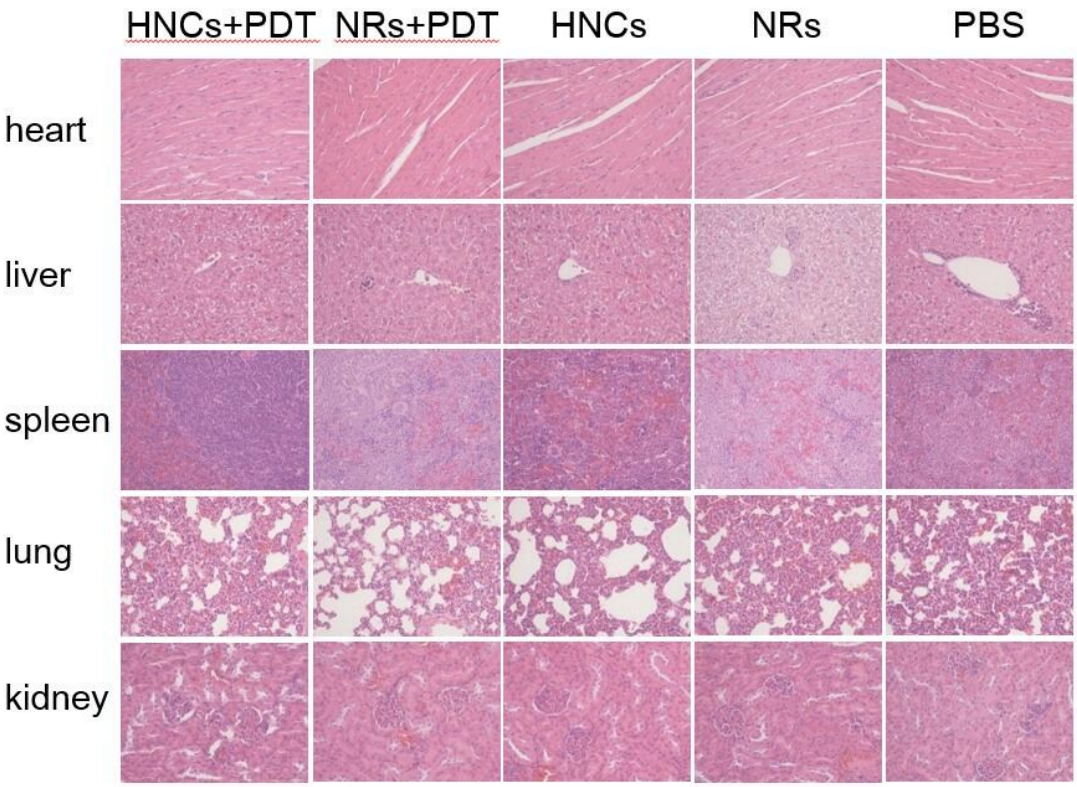

Figure S9. H&E-stained slices of major organs (heart, liver, spleen, lung and kidney) in mice after different treatments for 16 days.

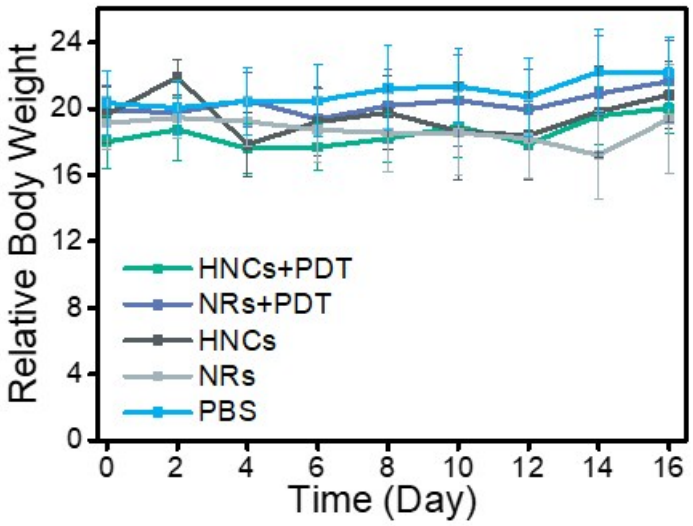

Figure S10. Relative body weights of the different treatment groups.

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